## Research funded by stem cell agency leads to novel fertility treatment, one child born

Posted: September 30, 2013

Research funded by California's stem cell agency has resulted in a novel way of treating some forms of infertility. One child has been born using the technique in Japan and another woman is now pregnant.

Aaron Hsueh, PhD, at Stanford University School of Medicine had received a Basic Biology grant from the agency, the California Institute for Regenerative Medicine (CIRM), to find a way of producing mature eggs from donated ovaries. His goal was to use these eggs in somatic cell nuclear transfer (SCNT), also known as therapeutic cloning, to create new stem cell lines for research and possible therapies.

Hsueh knew that in most cases donated ovaries still contain many thousands of tiny follicles containing immature eggs called oocytes. Girls are born with ovaries containing hundreds of thousands of these primitive follicles, one of which produces a mature egg each month after puberty. The challenge was to replicate that natural process of follicle maturation in ovaries from women who had donated them due to cancer or other reasons.

"We were taking ovarian tissue and trying to get mature oocytes. The goal was to do SCNT," said Hsueh, who is who is professor of obstetrics and gynecology and senior author of the study, published in the September 30 *Proceedings of the National Academy of Sciences*. "While doing this we found something surprising. We could activate follicles and they started to grow." He had found drugs that could prompt follicles to mature.

The recent study also built on what researchers had long-known: that disrupting the ovary could promote follicle growth in some infertile patients. Hsueh worked out the molecular underpinnings of this phenomenon, finding a set of proteins that act as a brake in the ovary to restrain follicle growth. They found that cutting the ovary into smaller pieces could overcome this brake.

Hsueh and his colleagues at St. Mariana University School of Medicine in Japan thought that combining the two techniques—breaking up the ovary then activating follicles using Hsueh's combination of drugs—might be an efficient way of producing eggs. They were right.

Although the work began as a way of producing eggs for SCNT, the group recognized another possible application for their discovery. Women with a condition called primary ovarian insufficiency (POI) go through menopause at a very young age and are then unable to get pregnant naturally. The condition is also called premature ovarian failure. The team thought their combined technique might help these women have children.

The team tested their approach in 27 women in Japan with POI. They removed ovaries from the women, fragmented the ovaries, and exposed the pieces to the drugs Hsueh had discovered. They then re-implanted ovary fragments near the fallopian tube of those women. Follicles in eight of the women showed signs of growing.

After that, the researchers gave hormones to the eight women to stimulate follicles to generate mature eggs. They were able to harvest eggs from five of the women. They fertilized the eggs in a lab dish using the husband's sperm and then implanted the several-day-old embryo into the woman's uterus. The group has called the technique in vitro activation, or IVA.

"This is an important development for young infertile women who do not ovulate their own eggs and are unable to have children of their own," said CIRM President Alan Trounson, PhD. Trounson was a pioneering scientist in the development of in vitro fertilization, which has since produced more than five million children. "The fact that this discovery resulted from a CIRM Basic Biology Award shows the value in funding basic science. Removing the inhibition of primary germ cells to grow in the ovary is an important development for reproductive medicine. We never know what important advances will result from scientists probing fundamental aspects of stem cell biology."

Kazuhiro Kawamura, MD, PhD, performed the caesarean section for the first child to be born using the technique; the child was in a breech position, which meant a normal delivery could have been dangerous to the baby and mother. Kawamura is associate professor of obstetrics and gynecology at the St. Marianna University School of Medicine and a lead author of the study. He also headed the

clinical aspects of the research.

“I could not sleep the night before the operation, but when I saw the healthy baby, my anxiety turned to delight,” Kawamura told Stanford University. “The couple and I hugged each other in tears. I hope that IVA will be able to help patients with primary ovarian insufficiency throughout the world.”

The Japanese clinicians say that the baby appears normal and healthy. In addition to helping the roughly 1% of women of reproductive age who are infertile due to POI, the researchers say the work could also help middle-age infertile women between 40 and 45 years of age, or cancer survivors who are infertile due to chemotherapy or radiation.

As for SCNT, Hsueh says that is no longer a focus of his lab. A team at Oregon Health & Science University succeeded in generating stem cell lines using SCNT last May. Hsueh is now hoping to improve IVA to make removing the woman's ovary unnecessary.

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